



Research Article

Risk Assessment of Pesticide Residues in Cauliflower Grown in Vicinity of Multan City, Pakistan

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Abstract | Pesticides are indispensable for successful vegetable production; however, misuse of insecticides results in chemical pollution and entry into the food chain. This study uniquely addresses the quantification of pesticide residues in cauliflower curds and soil, and associated human health risks in the specific climatic conditions of Multan City, and it is the first to collectively examine these five (lufenuron, bifenthrin, emamectin benzoate, metalaxyl, and mancozeb) pesticides in cauliflower. A survey of the cauliflower production area was performed to collect information about pesticides used for insect pest and disease management. Farmers were applying lufenuron, bifenthrin, emamectin benzoate, metalaxyl, and mancozeb. The cauliflower plant and soil samples were collected with the frequency of 1, 3, 5, 7, and 15 days after the application of pesticides. The collected plant and soil samples dried and extracted to determine pesticide residues using the modified QuEChERS method. Pesticides residues assessment was performed on High performance liquid chromatography (HPLC) at the Pesticide Quality Control Laboratory, Multan. There were no pesticide residues were detected in the soil samples. While in the cauliflower 20% samples (out of 40 samples) contained pesticide residues. Initial deposits of lufenuron of 0.93, 3.19, and 5.63 ppm were detected in the cauliflower samples of day 1 (after pesticide application) from the fields of farmers 1, 4, and 8, respectively. Bifenthrin residues of 1.64 and 1.78 ppm were detected in the cauliflower samples of day 1 (after pesticide application) from the fields of farmers 1 and 8, respectively. Similarly, bifenthrin residues of 0.81 and 0.61 ppm were detected in the cauliflower samples of day 3 (after pesticide application) from the fields of farmers 1 and 8. Bifenthrin residues were also detected in the 5th day sample (0.41 ppm) from the fields of farmer 8. While performing the risk assessment it was revealed that there will be no health risk associated for an average body weight (60 kg) person with the cauliflower consumption.

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Introduction

In developing countries, the increasing population necessitates higher agricultural production, which relies on effective crop protection techniques, primarily chemical pesticides. In order to cope with insect and pest attacks, many farmers prefer chemical control like pesticides/insecticides for their ease of control. The higher yield achieved by protecting the crops from pre-harvest and post-harvest insect-pest attacks necessitates increased use of these pesticides.

In addition, with the use of these pesticides not only ensures a consistent and cost-effective supply of produce, but also plays a crucial role in maintaining and boosting food production. However, while pesticides play a fundamental role in producing vast quantities of fruits and vegetables at a reduced cost, they are not devoid of drawbacks (Kin and Huat, 2010; Bidari *et al.*, 2011; Banshtu *et al.*, 2018). The consistent deployment of these toxic substances brings consequential side effects, making it crucial to evaluate and strike a balance between volume-driven agricultural practices and health considerations. According to the Ministry of National Food Security and Research of Pakistan, the area under cauliflower production and its yield has shown fluctuations over the past few years, in 2021-22 cauliflower was cultivated on 275984 hectares with the production of 11155 tones (GOP, 2023).

Cauliflower is infested by many insects, pests and diseases (Banshtu *et al.*, 2018). Cauliflower faces many insect-pests like tobacco caterpillar (*Spodoptera litura* F.), cabbage head borer (*Hellula undalis*), diamondback moth (*Plutella xylostella*), green peach aphid *Myzus persicae* (Sulzer), cutworm (*Varies*), corn earworm (*Helicoverpa zea*), cabbage maggot (*Delia radicum*), cabbage looper (*Trichoplusia ni*), etc. (Harcourt, 1966; Ahuja *et al.*, 2012; Sharma and Rao, 2012; Ghimire *et al.*, 2020). Similarly, there are many diseases that attack cauliflower, like alternaria leaf spot, damping off, black rot, clubroot, etc. (Mazzucchi and Fabianelli, 1970; Alvarez and Cho, 1978; Ahuja *et al.*, 2012; Botero *et al.*, 2019).

The extensive applications of pesticides to control insect-pests and diseases but they lead to their accumulation in soil, where they persist and affect soil health (Mandal *et al.*, 2020). Bifenthrin residues were detected in the soil with the mean recovery within the

range of 82-96.67% (Meena *et al.*, 2022). In another study, 0.082, 0.019, 0.018, 0.016, 0.0067, 0.0014 and 0.0007 mg/kg residues of malathion, cyproconazole, propargite, butachlor, chlorpyrifos, diazinon, and imidacloprid were detected in the soil samples, respectively (Mahdavi *et al.*, 2024).

Applying pesticides to cauliflower for extensive protection against insect pests and diseases can be envisioned. This practice significantly affects the quantity and quality of cauliflower curds. Farmers often resort to repeated and high-dose pesticide applications to safeguard their crops. Consequently, this excessive and intensive insecticide use has contributed to insect pest resistance and pesticide residue accumulation in cauliflower (Brar *et al.*, 2017). Because of this, extensive application of pesticides on the cauliflower, this study was designed to determine the pesticide residues in the cauliflower curds and soil samples, and health risks associated with the consumption of contaminated cauliflower. The hypothesis of the current study was that the misuse of pesticides leaves serious/significant levels of residues in vegetables and soil. The main objective of the study was to conduct a quantitative analysis of pesticides residues in cauliflower curds and soil, and conduct a risk assessment analysis. This study uniquely addresses the quantification of pesticide residues in cauliflower curds and soil, and associated human health risks in the specific climatic conditions of Multan city.

Materials and Methods

Field survey

A survey was conducted in the vicinity of Multan District (Figure 1) regarding the cauliflower production and the pesticides being used on it as observed in Table 1. A questionnaire was designed for this purpose.



Figure 1: Selected farmers' fields.

Table 1: Pesticides applied by the selected farmers.

	Lufenu-ron	Emamectin benzoate	Bifenthrin	Metalaxyl + Mancozeb
Farmer 01	✓	✓	✓	
Farmer 02	✓			
Farmer 03	✓			✓
Farmer 04	✓	✓	✓	
Farmer 05	✓			✓
Farmer 06	✓			
Farmer 07	✓	✓	✓	✓
Farmer 08	✓	✓	✓	✓

Sampling

Sampling of cauliflower curd and soil was performed at the intervals of 1, 3, 5, 7, and 15 days after the application of pesticides by the farmers, and labelled as presented in [Table 2](#).

Extraction protocol for plant samples

The modified Quick Easy Cheap Effective Rugged Safe Method (QuEChERS) method was used for the extraction. Cauliflower curds were homogenized

ground to a fine homogenized powder using a standard food processor and homogenizer. Fifteen grams of homogenized ground cauliflower curds sample was taken in a 50 ml centrifuge tube. Then, 15 ml of 1% acetic acid in acetonitrile (HPLC grade) was added to the tube. Tubes was capped and shaken by the hand for one minute. Then 6g of anhydrous magnesium sulfate and 1.5g anhydrous sodium acetate was added. After being capped, tubes were shaken vigorously by hand, followed by centrifugation for 5 minutes at 4000 rpm. A 8 ml aliquot of upper acetonitrile layer was transferred into another polypropylene tube with the capacity of 15 mL containing 1.2 g magnesium sulfate and 0.4 g Primary Secondary Amines (PSA). Then, the polypropylene tubes were capped tightly and shaken for 1 minute, followed by centrifugation for 5 minutes at 4000 rpm. Then, the 1.5 ml extract was transferred to the vial after filtering it with syringe filter (0.22 µm) for analysis ([Kusvuran et al., 2012](#)). Then the samples were analyzed through the HPLC with photodiode array detector (PDA) and variable wavelength detector (VWD) with the operating conditions stated in the [Table 3](#).

Table 2: Dates of samples collection after spray of pesticides at eight farmers sites.

	Day 01	Day 03	Day 05	Day 07	Day 15
Site 01	Nov 10, 2023	Nov 12, 2023	Nov 14, 2023	Nov 16, 2023	Nov 24, 2023
Site 02	Nov 02, 2023	Nov 04, 2023	Nov 06, 2023	Nov 08, 2023	Nov 16, 2023
Site 03	Oct 28, 2023	Oct 30, 2023	Nov 01, 2023	Nov 03, 2023	Nov 11, 2023
Site 04	Nov 10, 2023	Nov 12, 2023	Nov 14, 2023	Nov 16, 2023	Nov 24, 2023
Site 05	Oct 29, 2023	Oct 31, 2023	Nov 02, 2023	Nov 04, 2023	Nov 12, 2023
Site 06	Nov 04, 2023	Nov 06, 2023	Nov 08, 2023	Nov 10, 2023	Nov 18, 2023
Site 07	Nov 06, 2023	Nov 08, 2023	Nov 10, 2023	Nov 12, 2023	Nov 20, 2023
Site 08	Nov 06, 2023	Nov 08, 2023	Nov 10, 2023	Nov 12, 2023	Nov 20, 2023

Table 3: Operating conditions of HPLC with PDA and VWD.

Operating conditions	Lufenuron	Bifenthrin	Emamectin benzoate	Metalaxyl	Mancozeb
Instrument	HPLC-PDA				HPLC-VWD
Mobile phase	ACN:H ₂ O 70:30	ACN:H ₂ O 90:10	ACN:MeOH:H ₂ O 640:300:300) + 4 drops of TEA	ACN:H ₂ O 80:20	Amonium For- mate : Metha- nol 97.5:2.5
Column	C-18 (150 mm × 4.6 mm)	C-18 (150 mm × 4.6 mm having 5 µm particles)	C-18 (150 mm × 4.6 mm having 5 µm particles)	C-18 (250 mm × 4.6 mm)	C-8
Flow rate	1.5 ml	1.2 ml/min	1.5 ml/min	1.0 ml/min	1 ml/min
Wavelength	255 nm	240 nm	254 nm	230 nm	285 nm
Column Temp.	30 °C	40 °C	30 °C	Ambient	Ambient
Injection Vol.	5 µl	5 µl	10 µl	10 µl	5 µl
Retention time	8 min	12 min	15 min	10	10
Run time	15 min				
Diluent for standard	ACN	ACN	5 ml of MeOH + Mobile phase	ACN	EDTA+ Amo- nium formate
Elution	Isocratic				Gradient

Extraction protocol for soil samples

Initially, soil was dried, sieved and then 10±0.5 g were weighted and transferred into 50 ml centrifuge tube. Then 10 ml of 2.5% formic acid in acetonitrile (HPLC grade) was added to the tube. Next, 6 g and 1.5 g gram of anhydrous magnesium sulfate and sodium acetate was added in the tube respectively and shaken for a minute and sonicated for 15 minutes. Then, the samples were subjected to rotatory shaker for 25 minutes. After that they were centrifuged for 10 minutes at 4200 rpm. An aliquot of supernatant extract will be filtered (0.22 µm syringe filter) (Acosta-Dacal *et al.*, 2021) and analysed through HPLC with PDA and VWD with the operating conditions stated in the Table 3.

Health risk assessment methodology

In line the approach presented by (Tarawneh *et al.*, 2019) for the purpose of assessing the risk associated with the consumption of produce containing pesticide residues, first of all estimated average daily intake (EADI), will be calculated using the Equation 1.

$$EADI = F \times R \times P \dots(1)$$

Where, F and R show the consumption rate of the cauliflower in kg/day and the residue level of the pesticide present in ppm. P indicates the processing factor and taken as 1. The consumption rate of cauliflower in Pakistan is 0.04545 kg/day (45.45 g/day) (Arifullah *et al.*, 2008; Talat *et al.*, 2022).

The intake by a person per body weight, will be calculated by using the Equation 2, considering 60 kg as average adult body weight.

$$Intake = EADI / Body\ weight \dots(2)$$

Then we will find the hazard risk index (HRI) by using Equation 3, which is necessary to conduct human health risk assessment. HRI is actually a ratio between intake per body weight and acceptable daily intake (ADI) values. ADI is 0.02 and 0.01 (mg/kg bw/day) in case of lufenuron and bifenthrin, respectively (FAO-WHO, 2009, 2015).

$$HRI = Intake / ADI \dots (3)$$

Results and Discussion

Pesticide residues detection

The concentration of pesticide residues was calculated

using the Equations 4 and 5 presented by (Khan *et al.*, 2020). Where first of all we will calculate the response factor which is the ratio of the peak area of standard and standard amount or concentration of standard. Later we will calculate the concentration of residues by dividing peak area of sample with the response factor. There were no pesticide residues were detected in the soil samples while pesticide residues were detected in the cauliflower curds samples. Pesticide residues were detected in the 20% samples (out of 40 samples) (Table 4 and Figure 2). Only the residues of lufenuron and bifenthrin, from the pesticides applied on the cauliflower, are mentioned in the Table 4. All of them exceed the MRL which were 0.01 ppm for lufenuron (EU, 2018a) and 0.4 ppm for bifenthrin (EU, 2018b).

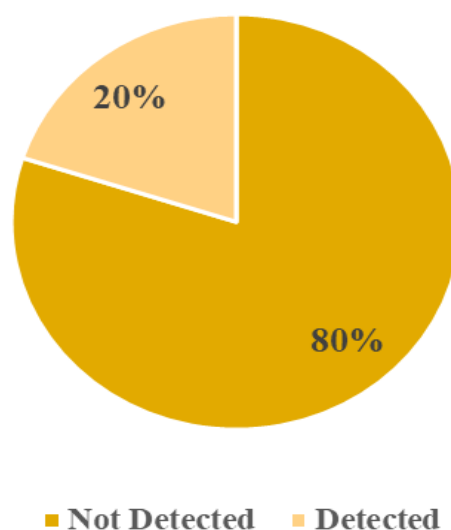


Figure 2: Pesticide residues detected in cauliflower curds samples.

Table 4: Detected pesticide residues in cauliflower samples.

Pesticide	Farmers	Day	Pesticide residue level (ppm)	MRL (ppm)
Lufenuron	Farmer 01	1	0.93	0.01
	Farmer 04	1	3.19	
	Farmer 08	1	5.63	
Bifenthrin	Farmer 01	1	1.64	0.4
	Farmer 01	3	0.81	
	Farmer 08	1	1.78	
	Farmer 08	3	0.61	
	Farmer 08	5	0.41	

Initial deposits (day 1) of the lufenuron in the fields of farmers 1, 4 and 8 were 0.93, 3.19, and 5.63 ppm, respectively. While there were no residues of lufenuron were detected in the subsequent samples on

Table 5: Health risk estimation for the detected pesticides in cauliflower samples.

Pesticides	Farmers	Days	Pesticide residue level (ppm)	EDAI	Intake per body weight	ADI	HRI	Health risk
Lufenuron	Farmer 01	1	0.92631	0.0421	0.000701677	0.02	0.0351	NO
	Farmer 04	1	3.19363	0.1452	0.002419175		0.1210	NO
	Farmer 08	1	5.63209	0.2560	0.004266306	0.2133	NO	
Bifenthrin	Farmer 01	1	1.64072	0.0746	0.001242845	0.01	0.1243	NO
	Farmer 01	3	0.81057	0.0368	0.000614006		0.0614	NO
	Farmer 08	1	1.77548	0.0807	0.001344925		0.1345	NO
	Farmer 08	3	0.61477	0.0279	0.000465685		0.0466	NO
	Farmer 08	5	0.40898	0.0186	0.000309802		0.0310	NO

day 3, 5, 7, and 15. And the initial deposits (1 day after pesticide application) of bifenthrin were detected in the samples from the fields of farmer 1 and 8 that were 1.64 and 1.78 ppm, respectively. While in 3rd day samples residues were 0.81 and 0.61 ppm, respectively. Bifenthrin residues were also detected in the 5th day sample (0.41 ppm) from the fields of farmer 8 but there were no residues detected in the samples from the fields of farmer 1.

$$\text{Response factor} = \frac{\text{peak area of standard}}{\text{standard amount}} \dots(4)$$

$$\text{Amount of analyte} = \frac{\text{peak area of sample}}{\text{response factor}} \dots(5)$$

Health risk assessment

Human health risk assessment regarding the consumption of contaminated cauliflower was performed by using the formulas (Equations 1, 2, and 3) and method presented by (Tarawneh *et al.*, 2019). Results of the human risk assessment are presented in the Table 5. Although, all the samples were exceeding their respective MRLs but still there was no health risk associated when the human health risk assessment analysis was performed because hazard risk index (HRI) was below 1 in all samples for a person with an average weight of 60 kg with the cauliflower consumption rate of 45.45 g/day (Arifullah *et al.*, 2008; Talat *et al.*, 2022). When there is HRI below 1 there will be no human health risk associated (Tarawneh *et al.*, 2019). There are several studies like Mujahid *et al.* (2022) and Jia *et al.* (2019) which have reported the residues above MRLs but still have no associated health risks.

Conclusions and Recommendations

The overuse of agro-chemical (like pesticides) is increased in the agriculture sector in order to enhance

the production to get more economical benefits, feed more people, and cope with the insect pest attack. This is one positive half of the story. The other half of the story shows that the pesticide can be deposited in the fruits and vegetables can have the adverse effects on human health and the environment. This study reveals the presence of pesticide residues in the cauliflower and exceeding the MRLs and no residues were detected in the soil. Out of 40 samples 20% samples contained residues. In this study, lufenuron and bifenthrin residues were detected in cauliflower samples. Beside the detection of lufenuron and bifenthrin residues but they still have not associated health risks for a person with the average weight of 60kg because the HRI was under 1 for all the contaminated samples. This study showed no health risks even residues concentration exceeds MRLs, but to be on the safer side if we will consume cauliflower after 5th day of pesticide application will eliminate any possible residues consumption because in this study residues were detected till the 5th day of pesticide application. Based on the findings, it is recommended to implement the effective monitoring programs in the vegetable growing areas. For policymakers, this means establishing regular pesticide residue testing and enforcing regulations to ensure compliance with safety standards. Furthermore, policymakers spread awareness about the methods to reduce the residues contents in the vegetables, like washing, boiling, and frying/cooking (Randhawa *et al.*, 2007, 2014; Kar *et al.*, 2012; Panhwar and Sheikh, 2013; Jia *et al.*, 2019; Vijay *et al.*, 2024) can residues the residue levels in cauliflower, and the treatments like 5% sodium bicarbonate aqueous (Vijay *et al.*, 2024) can be effective to reduce the residues buildup.

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Novelty Statement

This study uniquely addresses the quantification of pesticide residues in cauliflower curds and soil, and associated human health risks in the specific climatic conditions of Multan City, Pakistan.

Author's Contribution

Muhammad Nauman Hanif, Tanveer-ul-haq and Muhammad Naeem Akhtar: Planned the research, data analysis and wrote this MS.

Abib Hussain, Tanveer-ul-Haq and Amar Matloob: Supervised the study and revised the final draft.

Conflict of interest

The authors have declared no conflict of interest.

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